United States Department of the Interior
U. S. GEOLOGICAL SURVEY
Columbia Environmental Research Center
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Date: October 10, 2012

To: Ed Hammer, USEPA Region 5, Chicago, IL

From: Chris Ingersoll and Ed Little

Subject: USGS Columbia Environmental Research Center (USGS-Columbia) quarterly project summary for the project entitled: "Protectiveness of water or sediment quality guidelines to species of special concern"

Attached please find the 07/01/12 to 09/30/12 quarterly project summary for USGS-Columbia project entitled: “Protectiveness of water or sediment quality guidelines to species of special concern.” Please contact us if you have any questions concerning the attached summary (573/876-1819, fax -1896, email cingersoll@usgs.gov, elittle@usgs.gov). Please let us know if there are other individuals that should be receiving our quarterly summaries.

This quarterly summary provides progress on the following 5 tasks associated with the project:

A. Task 1. Determining the acute and chronic toxicity of ammonia, nitrate and nitrite to amphibians (USGS lead investigator: Ed Little)
B. Task 2. Developing and demonstrating a sediment toxicity test method with freshwater mussels for assessing sediment contaminants in the Great Lakes Basin and within North America
(USGS lead investigator: Chris Ingersoll)
C. Task 3. Evaluating of conditions used to culture or conduct toxicity tests with the amphipod *Hyalella azteca* (USGS lead investigator: Chris Ingersoll)
D. Task 4. Assessing the toxicity of sulfate in water to early life stages of fish, snails, and mussels (USGS lead investigator: Ning Wang)
E. Task 5. Improving concordance of sediment chemistry and toxicity (technical assistance to the USEPA Great Lakes National Program Office; USGS lead investigator: Chris Ingersoll)
F. Task 6. Water-only toxicity testing of snails and mussels (USGS lead investigator: Ning Wang)
G. Task 7. Major ion toxicity testing with select toxicity organisms (A: Influence of water hardness on the chronic toxicity of sulfate and chloride to fathead minnows (*Pimephales promelas*) and cladocerans (*Ceriodaphnia dubia*) and B: Acute and chronic toxicity of potassium to select aquatic organisms) USGS lead investigators: Ning Wang and Chris Ingersoll)
H. Task 8. Water-only toxicity testing with sculpin and darters (USGS lead investigator: John Besser; Task not funded by EPA, but being conducted by USGS support in ongoing

GLRI studies)

cc: Ning Wang, Nile Kemble, John Besser, Bethany Williams, Carl Orazio, Rip Shively, Norman Grannemann (USGS)
Task 1:Chuck Stephan
Task 2 and 6: Chris Barnhart, Missouri State University
Task 3: Dave Mount, Russ Hockett, Chuck Stephan (USEPA), Lisa Taylor, Warren Norwood (Environment Canada), Dave Soucek (INHS)
Task 4, 7, 8: Chuck Stephan (USEPA), Dave Soucek (INHS), Mike Coffee (USFWS)
Task 5: Scott Ireland, Dave Mount (USEPA)

COLUMBIA ENVIRONMENTAL RESEARCH CENTER
UNITED STATES GEOLOGICAL SURVEY, COLUMBIA, MO
QUARTERLY PROGRESS SUMMARY

USGS Basis+ Program Element 42100, USGS Basis+ Project 2145E52 (USGS template/task 240 GLNPO sediment; USGS template/task 251 Hyalella; USGS template/task 252 Sulfate; USGS template/task 253 Mussel sediment; USGS template/task 254: Amphibians; USGS template/task 147 Major ion toxicity)

USGS Project Managers: Chris Ingersoll and Ed Little

Title: Protectiveness of water or sediment quality guidelines to species of special concern

USGS-Columbia is developing quarterly summaries for the USEPA that are intended to describe the status of activities associated with the seven following tasks associated with collaborative research between USGS and USEPA to evaluate the protectiveness of water or sediment quality guidelines to species of special concern (Interagency agreement between USEPA and USGS dated February 1, 2010).

A. Task 1. Determining the acute and chronic toxicity of ammonia, nitrate and nitrite to amphibians (USGS lead investigator: Ed Little)
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(USGS lead investigator: Chris Ingersoll)
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hardness on the chronic toxicity of sulfate or chloride to fathead minnows (*Pimephales promelas*) and cladocerans (*Ceriodaphnia dubia*) and B: Acute and chronic toxicity of potassium or calcium to select aquatic organisms) USGS lead investigators: Ning Wang and Chris Ingersoll)

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1. What work was accomplished for this past quarter (07/01/12 to 09/30/12)?

A. Task 1. Determining the acute and chronic toxicity of ammonia, nitrate and nitrite to amphibians

1. Continued to culture southern two-lined salamander (*Eurycea cirrigera*) and mudpuppy (*Necturus maculosus*) larvae, including transitioning mudpuppies to feeding. Maintained two-lined salamanders in culture until the onset of metamorphosis, demonstrating the ability to culture this species from egg through the full larval period.
2. Completed 30-day chronic exposures of larval *E. cirrigera* and *N. maculosus* to chloride (as NaCl) at 1000, 500, 250, 125, and 62.5 mg Cl/L. Control survival was near 100% for both species, demonstrating that an intermittent flow-through proportional diluter is a suitable system for conducting chronic toxicity tests with these infrequently tested organisms.
3. Acute (96-hour) exposures to chloride (as NaCl) are currently underway with both *E. cirrigera* and *N. maculosus*, evaluating whether static renewal conditions are suitable for acute tests with these species.
4. Completed preliminary acute tests in the flow-through diluter system with both *E. cirrigera* and *N. maculosus* exposed to ammonia (NH_4Cl).
5. Participated in CERC/NAWQA meeting on August 10, 2012 to further discuss logistics of and site selection for amphibian tests associated with NAWQA Cycle III Regional Synoptic Study.

B. Task 2. Developing and demonstrating a sediment toxicity test method with freshwater mussels for assessing sediment contaminants in the Great Lakes Basin and within North America

1. USGS personnel participated in scheduled conference calls with USEPA and other interested groups to discuss the status of research conducted and planned associated with this task.
2. Developed a plan to conduct additional 28-d sediment toxicity testing with fatmucket (*Lampsilis siliquoidea*). The source of the sediment will be dilutions of a highly contaminated sediment collected under the direction of Scott Ireland from the East Branch of the Grand Calumet River (Task 5). The goal of this next study will be to compare responses of various life stages of amphipods or midge (e.g., 4- vs 7-day old organisms measuring survival, weight, biomass, or reproduction for amphipods or survival, weight, biomass, or emergence of midge) to the responses of mussels (e.g., 28-d survival, weight, biomass).
3. Finalized a design of sediment chambers for evaluating the behavior of various ages of mussels placed in sediment (see photo in Attachment 1). A total of 5 mussels and 40 mm of sediment with 20 mm of overlying water is placed in a modified 60-ml

Monoject syringe (the top 5 mm of the syringe is cut away). At a sampling time period, sediment is extruded out the top of the syringe and thin slices of sediment are isolated to find burrowing mussels by depth in the sediment. The thinnest slice of sediment that is practical to isolate from the syringe is about 1.8 mm. Hence, about 20 slices of sediment with a thickness of about 1.8 mm can be collected from a 40-mm depth of sediment.

4. Burrowing depth of two ages of juvenile fatmucket (*Lampsilis siliquoidea*) was monitored after introduction into the sediment chambers at 4-hour and 24-hour intervals in three control sediments (West Bearskin [WB], Spring River [SR] and Culture Sand [CS]). All of the larger mussels burrowed into the sediment within 15 minutes of being introduced into the syringe (smaller mussels were difficult to observe immediately after introduction at the sediment-water). After 24 hours, about 75% of the younger mussels (2-weeks post transformation) were found within the upper 1.8 mm of the sediment in both the WB and CS sediments with maximum burrowing depth of 3.6 mm. Similarly, 74% of the older mussels (10-weeks post transformation) were found within the upper 1.8 mm of the sediment at 4 hours and at 24 hours with a maximum burrowing depth of 7.2 mm. Additional studies are planned with different life stages of mussels and different species of mussels to evaluate burrowing behavior in control sediment(s) or in contaminated sediments. Anecdotal observations indicate that about 6-month-old fatmucket juveniles no longer burrow into sediment, but instead are filtering water and particles at the sediment-water interface.

C. Task 3. Evaluating of conditions used to culture or conduct toxicity tests with the amphipod *Hyalella azteca*
    1. USGS personnel participated in weekly conference calls with USEPA and other interested groups to discuss the status of research conducted and planned associated with this task and planned revisions to USEPA and ASTM methods based on the findings from these studies.
    2. Refined USGS methods for culturing known-age amphipods and midge (e.g., to start exposures with 4-d-old vs 7-d-old amphipods or midge). See Task B2 for additional details.
D. Task 4. Assessing the toxicity of sulfate in water to early life stages of fish, snails, and mussels
    1. USGS personnel participated in scheduled conference calls with USEPA and other interested groups to discuss the status of research conducted with this task.
    2. Ning Wang and Chris Ingersoll attended an August 22, 2012 meeting with USEPA Region 5 and USFWS in Moline IL to discuss findings from this study and plans for future studies (see Task 7).
E. Task 5. Improving concordance of sediment chemistry and toxicity (technical assistance to the USEPA Great Lakes National Program Office)
    1. USGS personnel participated in scheduled conference calls with USEPA and other interested groups to discuss the status of research conducted and planned associated with this task and planned revisions to USEPA and ASTM methods based on the findings from these studies.
    2. Developed a plan for evaluating the response of different life stages of amphipods or midge (e.g., <24-h, 4-d vs 7-day old organisms) with exposure to dilutions of a highly

contaminated and toxic sediment sample collected from the East Branch of the Grand Calumet River. The objective of this next study will be to determine if exposures started with 4-d-old midge improves control performance or if exposures started with 4-d-old amphipods will result in a delay of reproduction past Day 28 of a sediment exposure. Improved feeding methods in water exposures started with about 7-d-old amphipods has resulted in improved growth and reproduction starting before Day 28. Hence, for sediment exposures with amphipods where reproduction is to be determined, it may be desirable to start with younger amphipods (4-d-old rather than 7-d-old) to delay the onset of reproduction past the end of the 28-d sediment exposure.

F. Task 6. Water-only toxicity testing of snails and mussels (USGS lead investigator: Ning Wang)
    1. Finalized list of the ten Tier 1 chemicals and species for conducting water-only acute toxicity testing with snails and five tribes of mussels.
    2. Developed a proposed list of ten Tier 2 chemicals for conducting acute water-only toxicity tests with fatmucket.
    3. Completed toxicity tests with two mussel species (fatmucket and western pearlshell) and started acute toxicity tests with white heelsplitter with the ten Tier 1 chemicals. The preliminary results indicate that (a) the different mussel tribes had similar sensitivity to chemicals, and (b) the tested mussels were very sensitive to potassium chloride but insensitive to malathion and metolachlor relative to other organisms in literature (e.g., US EPA ECOTOX Database).
    4. Received water samples for chemical analyses of inorganic Tier 1 and Tier 2 chemicals. Nominal and measured concentrations were in relatively close agreement, except for aluminum (a Tier 2 chemical). It is likely that the problem with aluminum is testing above solubility. We will likely select an alternate chemical to aluminum for Tier 2 testing
    5. Submitted water samples for chemical analyses of organic Tier 1 and Tier 2 chemicals.

G. Task 7. Major ion toxicity to select aquatic organisms (USGS lead investigators: Ning Wang and Chris Ingersoll).
    1. Task 7a: Sulfate or chloride toxicity influenced by water quality
        a. Completed methods development for conducting a short-term static-renewal toxicity test with fathead minnows starting with newly fertilized eggs, continuing through hatching (by about Day 6) to about 10 day after hatching. The preliminary results indicated the high rates of hatching, survival, and growth in control water under static-renewal conditions.
        b. Developed draft plan for conducting sodium sulfate toxicity tests with fathead minnows using the short-term static-renewal method.
    2. Task 7b: Potassium or calcium toxicity influenced by water quality
        a. A 96-h $CaCl_2$ toxicity test has been conducted with newly transformed juvenile fatmucket.

H. Task 8. Water-only toxicity testing with sculpin and darters (USGS lead investigator: John Besser)
    1. Previous acute and chronic tests with mottled sculpins (*Cottus bairdi*) indicated that current acute and chronic water quality criteria for cadmium, copper, and zinc may

not adequately protect populations of this widespread species (Besser et al. 2007; *Environ Toxicol Chem* 26:1657-1665).

2. We conducted 96-h acute toxicity tests with juvenile mottled sculpins reared from eggs collected southern Ohio (0.18 g wet weight; about 4 months post-hatch) and exposed to carbaryl and 4-nonylphenol. Preliminary estimates of LC50s (based on nominal chemical concentrations) were 4.2 mg/L for carbaryl and 0.42 mg/L for 4-nonylphenol. These LC50s would rank 11th (for carbaryl) and 17th (for 4-nonylphenol) of the 18 fish species tested by Dwyer et al. (2005; *Arch Environ Contam Toxicol* 48:143-154).

2. What problems (or sources of error) were encountered, if any?

None

3. If a problem was encountered, what action was taken to correct it?

Not applicable

4. What work is projected for the new quarterly activity?

A. Task 1. Determining the acute and chronic toxicity of ammonia, nitrate and nitrite to amphibians
   1. Continue summarizing data from wood frog and gray tree frog embryos exposed to NaCl, KCl, CaCl2, and Na2SO4 in 2012 tests.
   2. Participate in conference calls with USEPA to plan potential test designs for 2013 definitive tests with southern two-lined salamander larvae (*Eurycea cirrigera*) and/or mudpuppies (*Necturus maculosus*). Discuss the possibilities for testing earlier-stage larvae to evaluate life-stage-specific differences in sensitivity seen in previous tests with gray treefrogs (*Hyla versicolor*) and wood frogs (*Lithobates sylvaticus*).
   3. Continue discussions about study design for atrazine/nutrient exposures of southern leopard frogs (*Lithobates sphenocephalus*) or gray treefrogs (*Hyla versicolor*) in association with NAWQA Cycle III Regional Synoptic Study.

B. Task 2. Developing and demonstrating a sediment toxicity test method with freshwater mussels for assessing sediment contaminants in the Great Lakes Basin and within North America
   1. Continue evaluating behavior of various species of early life stages of mussels in various sediments using the exposure system illustrated in Attachment 1.
   2. Identify additional sediments for evaluating mussel toxicity testing methods.
   3. Continue to develop plans for coordinating sediment testing of mussels as part of the USGS NAWQA agriculture project planned for the Midwest in 2013. This USGS NAWQA project will include sediment toxicity tests conducted with *H. azteca* or *C. dilutus* (information was provided to USEPA in an email dated September 28, 2012). The plan would be to test mussels in about 50 of the 100 sediments to be evaluated in 2013 by the USGS NAWQA program.

C. Task 3. Evaluating of conditions used to culture or conduct toxicity tests with the amphipod *Hyalella azteca*

1. Continue summarizing data for the four 42-d water-only test to evaluate the influence bromide or iodide on the response of *H. azteca* in reconstituted waters.
2. Continue developing plan with the HAAG for conducting inter-laboratory testing of the water-only *H. azteca* toxicity testing method.
3. Continue evaluating methods for culturing and testing known-age amphipod and midge (e.g., <24-h old, 4-d old, 7-d old). Conduct study to evaluate starting age of *H. azteca* or *C. dilutus* in control water or in control sediment using different diets.

D. Task 4. Assessing the toxicity of sulfate in water to early life stages of fish, snails, and mussels
   1. Continue drafting a manuscript for submission to a scientific journal describing the results of the acute and chronic sulfate toxicity testing. Timing of this manuscript will depend on the findings of studies conducted under Task 7.

E. Task 5. Improving concordance of sediment chemistry and toxicity
   1. Continue discussions with USEPA GLNPO regarding ongoing and future options for technical assistance associated with this task (e.g., additional sediments, comparisons of diets for H. *azteca* or *C. dilutus*, evaluate starting age of *H. azteca* in water or in sediment toxicity test [e.g., starting exposures with <24-h old or 4-d-old juveniles, rather than 7-day old juveniles]).
   2. Continue discussions with USEPA and Environment Canada on planned revisions to standard methods for conducting sediment or water toxicity tests with *H. azteca* or *C. dilutus*.

F. Task 6. Water-only toxicity testing of snails and mussels
   1. Conduct Tier 1 testing newly transformed Washboard (*Megalonaias nervosa*, Quadrulini tribe, to be cultured by the Genoa National Fish Hatchery).
   2. Estimate EC50 values once measured concentrations toxicants are available, and summarize preliminary data and prepare a poster for SETAC meeting in November.

G. Task 7. Major ion toxicity to select aquatic organisms (Task 7a: and Task 7b:)
   1. Task 7a: Sulfate or chloride toxicity influenced by water quality
      a. Conduct sulfate toxicity test with fathead minnows in three 100 mg/L hardness waters (100 hard well water [with about 10 mg Cl/L and about 20 mg SO_4/L], 100 hard well water with about 25 mg Cl/L (with addition of NaCl), and 100 hard ASTM (with about 93 mg sulfate in the base water).
      b. Exposures will be static-renewal toxicity tests conducted with fathead minnows starting with eggs and continuing for about 7 to 14 days after hatching. This study design would allow for testing more combinations of major ions and water quality characteristics compared to conducting approximately 35-day exposures starting with eggs of fathead minnows.
   2. Task 7b: Potassium or calcium toxicity influenced by water quality
      a. Summarize 96-h $CaCl_2$ toxicity data for the study conducted with newly transformed juvenile fatmucket.

H. Task 8. Water-only toxicity testing with sculpin and darters
   1. Summarized acute toxicity data and chemistry data from the preliminary acute toxicity tests conducted with sculpin.

5. Is the project work on schedule?

For the quarter? Yes
For the project? Yes

6. Does the project funding rate support the work progress?

Yes, given the revised budget period for Task 7 will continue to through June 2016.

7. What has been spent to date?

If requested, USGS will provide USEPA (or other interested groups) summaries of expenditures on the project.

8. Have you submitted a quarterly voucher for reimbursement?

USGS will provide vouchers as necessary.

9. Is there a change in principal investigator?

No

10. If you have a multi-year project with budget periods, have you submitted your request for a funding amendment?

Not applicable

Attachment 1. Design of the chambers (60-ml syringes) used to evaluate behavior of mussels in sediment.